

**Pcons-RBS-LO-GFP-  
His/pSB1C3**

Forward primer for sequencing/amplifying BioBrick parts (VF2)

Chloramphenicol resistance

rep (pMB1)

BioBrick Prefix

BBa J23101 (Pcons)

BBa B0034 (RBS)

BBa K1694035

Lpp-OmpA-BamHI

*Bam*HI (498)

*Nco*I (666)

BBa E0040 (GFP)

*Hind*III (1215)

*Ava*I (1230)

His-Tag coding sequence

T7T primer for sequencing

T7 terminator

BioBrick Suffix

*Spe*I (1387)

*Pst*I (1405)

Reverse primer for sequencing/amplifying BioBrick parts (VF1)

*Xba*I (3449)

*Eco*RI (3434)

*Ava*I (3310)

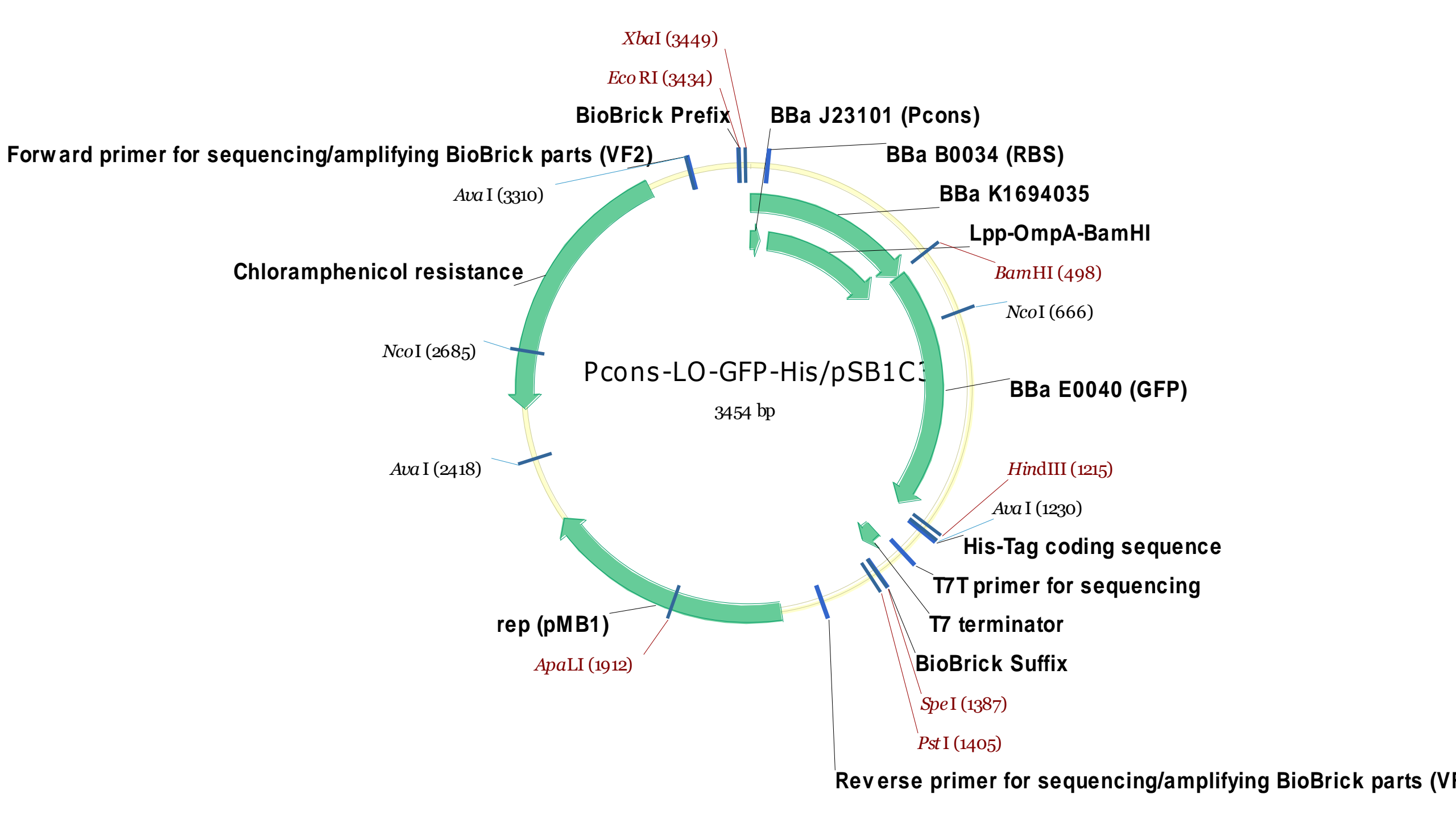
*Nco*I (2685)

*Ava*I (2418)

*Apa*LI (1912)

Pcons-LO-GFP-His/pSB1C3

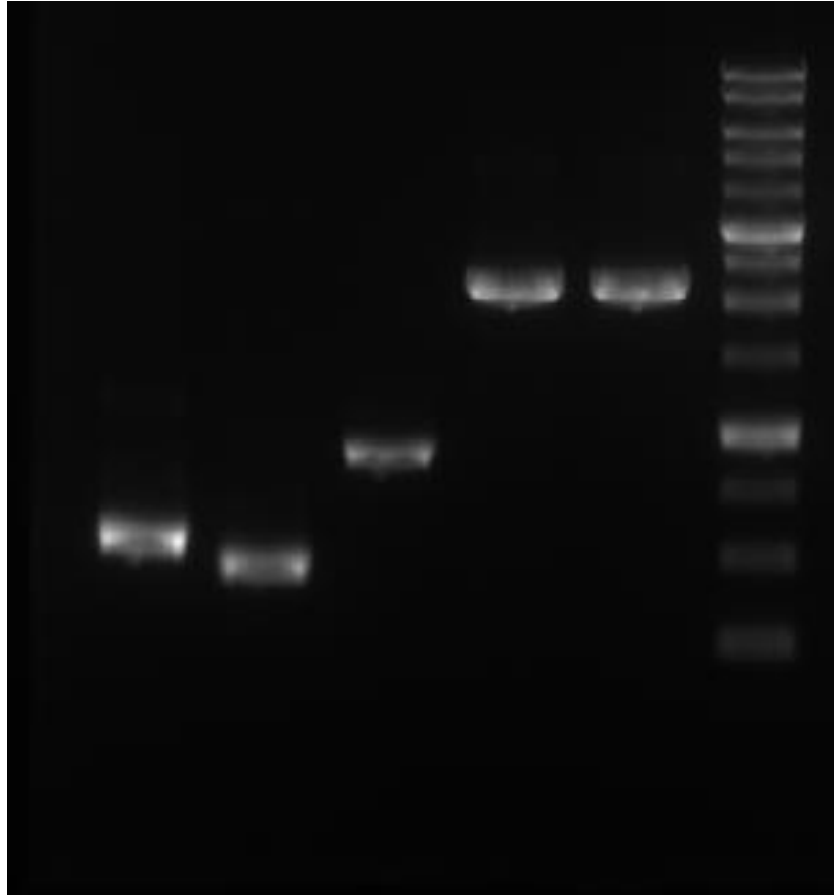
3454 bp



# Strategy

- The same as “CLONING - LO-GFP-His\_pSB1C3”

# PCR with KOD-plus



- Date: 2016.08.17

- Lane:

1. Pcons-RBS-LO-BamHI

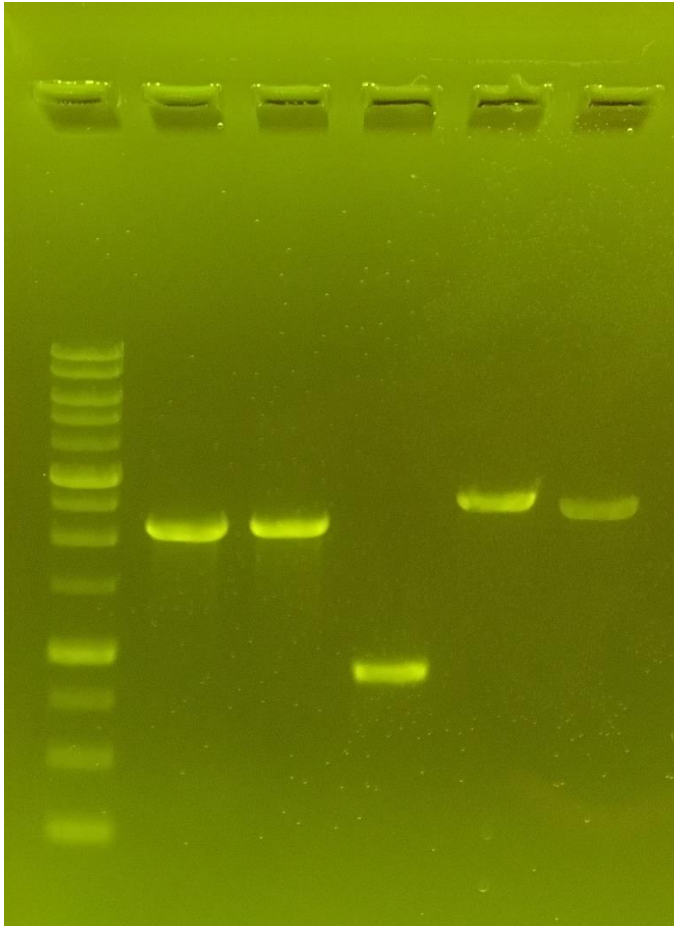
2. LO-BamHI

3. **GFP**

4. AOX1

5. AOX2

# Ligation



- Date: 2016.08.31

- Lane:

1. AOX1: BamHI + PstI

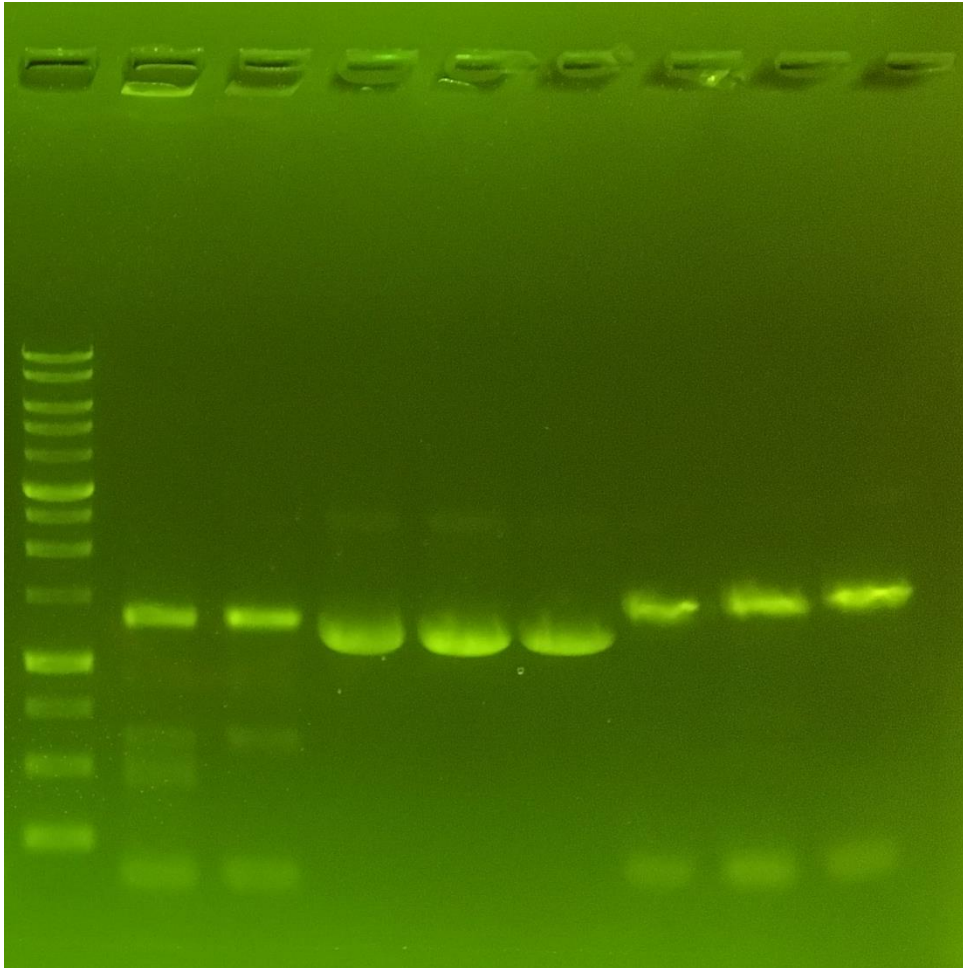
2. AOX2: BamHI + PstI

3. GFP: BamHI + PstI

4. Pcons-RBS-LO-BamHI/pSB1C3  
#P6: BamHI + PstI

5. LO-BamHI/pSB1C3 #L2: BamHI  
+ PstI

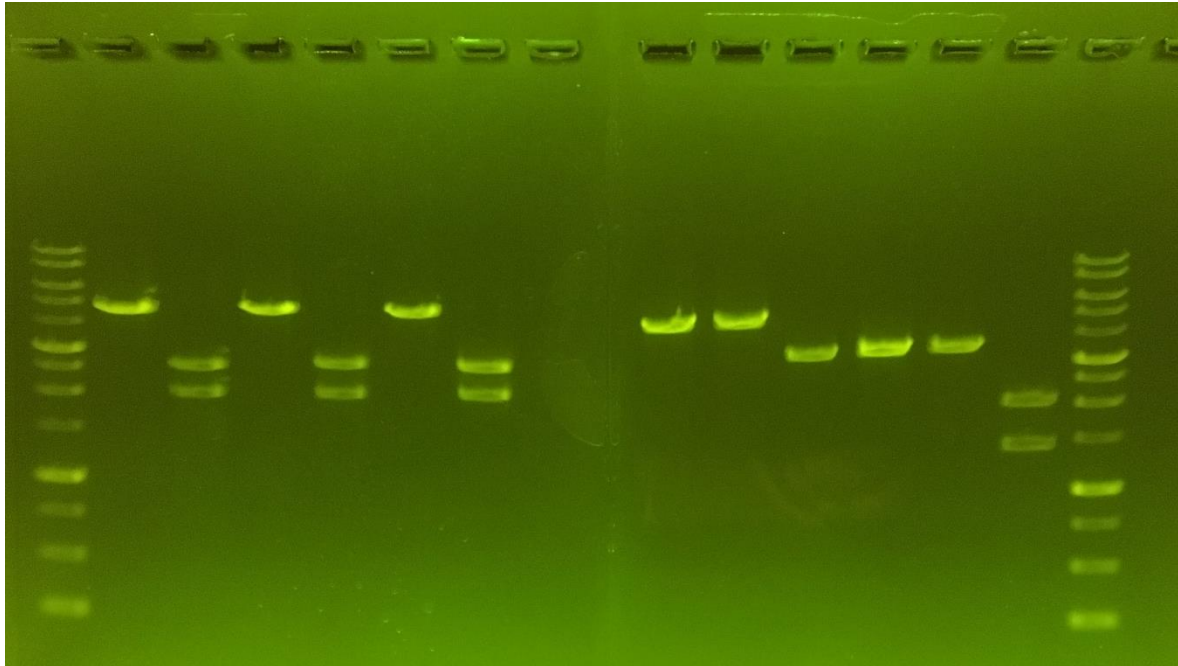
# Colony PCR



- Date: 2016.09.02
- Clone: Pcons-RBS-LO-GFP-His/pSB1C3
- Primer:
  1. Pcons-XbaI-EcoRI-F
  2. GFP-HindIII-R
- Lane: #PG-1 ~ #PG-3
- #PG-4 → Plasmid extraction → RE check

# RE check

- Date: 2016.09.07
- Clone: Pcons-RBS-LO-AOX2-His/pSB1C3
- Lane:



1. #L1-2: EcoRI
2. #L1-2: EcoRI + PstI
3. #P1-5: EcoRI
4. #P1-5: EcoRI + PstI
5. #L2-5: EcoRI
6. #L2-5: EcoRI + PstI
7. #P2-2: EcoRI (Failed)
8. #P2-2: EcoRI + PstI (Failed)
9. #LG-3: EcoRI (Failed)
10. #LG-3: EcoRI + PstI (Failed)
11. #PG-4: EcoRI
12. #PG-4: EcoRI + PstI

# Sequencing

- Date: 2016.09.14
- Sequencing primers: VF2, VR
- Clone: Pcons-RBS-LO-GFP-His/pSB1C3 #PG-4
- File: “2016.09.14\_Pcons-RBS-GFP\_pSB1C3 (#PG-4)”
- Result: correct → make a stock